

D1  
cont'd

Cont  
E3

(i) providing a cell, said cell comprising,

- a) a first heterologous promoter operably linked to a first polynucleotide encoding a functional G $\alpha$ 15 protein having at least 95 % sequence homology to SEQ. ID. NO. 2,
- b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene,
- c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR,

wherein said cell stably expresses said G $\alpha$ 15 protein at sufficient levels to permit promiscuous coupling to said GPCR,

wherein said GPCR is not naturally expressed in said cell, and

wherein said second heterologous promoter is directly or indirectly modulated by the activity of said G $\alpha$ 15 protein;

(ii) contacting said cell with said ligand; and

(iii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said ligand with reporter gene expression after addition of said ligand.

64. (Canceled)

65. (Canceled)

66. The method of claim 63, wherein said GPCR is a taste receptor.

67. (Amended) The method of claim 63, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenical acetyl transferase,  $\beta$ -galactosidase,  $\beta$ -lactamase and secreted alkaline phosphatase.

D2  
Sub 32

206 83  
68. The method of claim 63, further comprising contacting said cell with a compound that increases calcium levels inside said cell.

69. The method of claim 68, wherein said compound is selected from the group consisting of ionomycin and thapsigargin.

70. The method of claim 68, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

D3  
500 E4  
71. (Twice amended) A method for identifying a GPCR for a given ligand, the method comprising:  
i) providing a cell, said cell comprising,  
a first heterologous promoter operably linked to a first polynucleotide encoding a functional  $G\alpha_{15}$  protein having at least 95 % sequence homology to SEQ. ID. NO. 2, and  
a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR,  
wherein said cell stably expresses said  $G\alpha_{15}$  protein at sufficient levels to permit promiscuous coupling to said GPCR and wherein said GPCR is normally coupled to either  $G\alpha_i$ ,  $G\alpha_s$  or  $G\alpha_{12}$  in the absence of said  $G\alpha_{15}$  protein, and  
wherein said GPCR is not naturally expressed in said cell;  
ii) contacting said cell with said ligand; and  
iii) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said ligand with said signal after addition of said ligand, and  
wherein said signal transduction detection system comprises a dye.

72. (Canceled)

73. (Canceled)

50B  
F4

74. The method of claim 71, wherein said signal transduction detection system comprises an intracellular calcium indicator.

D4

75. (Twice amended) A method of identifying a ligand for a GPCR, the method comprising:

- 75B  
F5
- i) contacting a cell with a test chemical, said cell comprising,
    - a first heterologous promoter operably linked to a first polynucleotide encoding a functional G $\alpha$ 15 protein having at least 95 % sequence homology to SEQ. ID. NO. 2, and
    - a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR,
    - wherein said cell stably expresses said G $\alpha$ 15 protein at sufficient levels to permit promiscuous coupling to said GPCR and wherein said GPCR is normally coupled to either G $\alpha$ <sub>i</sub>, G $\alpha$ <sub>s</sub> or G $\alpha$ <sub>12</sub> in the absence of said G $\alpha$ 15 protein and,
    - wherein said GPCR is not naturally expressed in said cell;
  - ii) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said test chemical with said signal after addition of said test chemical,
- wherein said signal transduction detection system comprises a dye.

76. (Canceled)

77. (Canceled)

Sub F5

78. The method of claim 75, wherein said signal transduction detection system comprises an intracellular calcium indicator.

D5

79. (Twice amended) The method of claim 75, further comprising comparing a signal from a first plurality of cells in the presence of said test chemical with either:

05  
cont'd

- i) a signal from a second plurality of cells in the presence of said test chemical, wherein said second plurality of cells lack said Gα15 protein or
- ii) a signal from said first plurality of cells in the absence of said test chemical.

503  
EB

80. The method of claim 75, wherein said detecting comprises fluorescence detection.

06

81. (Twice amended) A method of identifying a ligand for a GPCR, the method comprising;

- i) contacting a cell with a test chemical, said cell comprising,
  - a) a first heterologous promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95 % sequence homology to SEQ. ID. NO. 2,
  - b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene,
  - c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR,wherein said cell stably expresses said Gα15 protein at sufficient levels to permit promiscuous coupling to said GPCR,  
wherein said GPCR is not naturally expressed in said cell,  
and  
wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein;
- ii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said ligand with reporter gene expression after addition of said ligand.

82. (Canceled)

83. (Canceled)

sub F7

84. The method of claim 81, wherein said detecting comprises fluorescence detection.

sub F7

85. (Amended) The method of claim 81, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenical acetyl transferase,  $\beta$ -galactosidase,  $\beta$ -lactamase and secreted alkaline phosphatase.

sub F9

86. The method of claim 81, further comprising contacting said cell with a compound that increases calcium levels inside said cell.

87. The method of claim 86, wherein said compound is selected from the group consisting of ionomycin and thapsigargin.

sub F7

88. The method of claim 81, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

08

89. (Twice amended) The method of claim 81, further comprising comparing a signal from a first plurality of cells in the presence of said test chemical with either:

- i) a signal from a second plurality of cells in the presence of said test chemical, wherein said second plurality of cells lack said G $\alpha$ 15 protein, or
- ii) a signal from said first plurality of cells in the absence of said test chemical.

sub F8

90. (Twice amended) A method for identifying a modulator of signal transduction mediated by GPCR activation in a cell, the method comprising:

- a) contacting a cell with a test chemical, said cell comprising,
  - a first heterologous promoter operably linked to a first polynucleotide encoding a functional G $\alpha$ 15 protein having at least 95 % sequence homology to SEQ. ID. NO. 2, and
  - a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR,

08  
cont'd

Cont  
E8

wherein said cell stably expresses said G $\alpha$ 15 protein at sufficient levels to permit promiscuous coupling to said GPCR and wherein said GPCR is normally coupled to either G $\alpha_i$ , G $\alpha_s$ , or G $\alpha_{12}$  in the absence of said G $\alpha$ 15 protein, and

wherein said GPCR is not naturally expressed in said cell;

- b) contacting said cell with a ligand that, in the absence of said test chemical, activates signal transduction via said GPCR in said cell, and
- c) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said test chemical with said signal after addition of said test chemical.

91. (Canceled)

92. (Canceled)

sub F10

93. The method of claim 90, wherein said signal transduction detection system comprises an intracellular calcium indicator.

09

94. (Twice amended) A method for identifying a modulator of signal transduction in a cell, the method comprising:

sub E9

- i) contacting a cell with a test chemical, said cell comprising,
  - a) a first heterologous promoter operably linked to a first polynucleotide encoding a functional G $\alpha$ 15 protein having at least 95 % sequence homology to SEQ. ID. NO. 2,
  - b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene,
  - c) a third heterologous promoter operably linked to a second polynucleotide encoding said GPCR,

wherein said cell stably expresses said G $\alpha$ 15 protein at sufficient levels to permit promiscuous coupling to said GPCR, and

Q9  
cont'd

Cont  
E9

wherein said second heterologous promoter is directly or indirectly modulated by the activity of said G $\alpha$ 15 protein, and wherein said GPCR is not naturally expressed in said cell;

- ii) contacting said cell with a ligand that, in the absence of said test chemical activates signal transduction via said GPCR in said cell; and
- iii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said test chemical with reporter gene expression after addition of said test chemical.

95. (Canceled)

96. (Cancelled)

Sub  
P11

97. The method of claim 94, wherein said detecting comprises fluorescence detection.

D10

Sub  
P12

98. (Amended) The method of claim 94, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenical acetyl transferase,  $\beta$ -galactosidase,  $\beta$ -lactamase and secreted alkaline phosphatase.

Sub  
P13

99. The method of claim 94, further comprising contacting said cell with a compound that increases calcium levels inside said cell.

100. The method of claim 99, wherein said compound is selected from the group consisting of ionomycin and thapsigargin.

Sub  
E10

101. The method of claim 94, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

D11

Sub  
E11

102. (Twice amended) A method of functionally profiling a test chemical comprising the steps of.

- i) contacting a panel of cells with a test chemical, said panel of cells

11  
cont'd

Cont  
E11

- comprising, a plurality of cell clones, each cell clone comprising
- a) a first heterologous promoter operably linked to a first polynucleotide encoding a functional G $\alpha$ 15 protein having at least 95 % sequence homology to SEQ. ID. NO. 2,
  - b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene,
  - c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR,
- wherein said cell stably expresses said G $\alpha$ 15 protein at sufficient levels to permit promiscuous coupling to said GPCR, wherein said second heterologous promoter is directly or indirectly modulated by the activity of said G $\alpha$ 15 protein,
- wherein said GPCR is not naturally expressed in said cell, and wherein each cell clone differs only with respect to said GPCR that is expressed;
- ii) contacting said cell clones with a test chemical;
  - iii) detecting reporter gene expression from said cell clones
  - iv) comparing reporter gene expression between said cell clones.

103. (Canceled)

104. (Canceled)

105. The method of claim 102, wherein said detecting comprises fluorescence detection.

sub F14

112

Sub  
315

106. (Amended) The method of claim 102, wherein said reporter gene is selected from the group consisting of luciferase, GFP, chloramphenical acetyl transferase,  $\beta$ -galactosidase,  $\beta$ -lactamase and secreted alkaline phosphatase.

112

107. The method of claim 102, further comprising contacting said cell with a



*but E12* compound that increases calcium levels inside said cell.

108. The method of claim 107, wherein said compound is selected from the group consisting of ionomycin and thapsigargin.
109. The method of claim 107, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

*D13*

110. (Amended) The method of claim 67, further comprising contacting said cell with a reporter gene substrate.

*7/16 E13*

111. (Amended) The method of claim 67, wherein said reporter gene is  $\beta$ -lactamase.

112. (Amended) The method of claim 85, further comprising contacting said cell with a reporter gene substrate.

*7/16 E14*

113. (Amended) The method of claim 85, wherein said reporter gene is  $\beta$ -lactamase.

114. (Amended) The method of claim 98, further comprising contacting said cell with a reporter gene substrate.

*7/16 E15*

115. (Amended) The method of claim 98, wherein said reporter gene is  $\beta$ -lactamase.

116. (Amended) The method of claim 106, further comprising contacting said cell with a reporter gene substrate.

117. (Amended) The method of claim 106, wherein said reporter gene is  $\beta$ -lactamase.

118. (Amended) The method of claim 110, wherein said reporter gene substrate is CCF2.

013  
cont'd

119. (Amended) The method of claim 112, wherein said reporter gene substrate is CCF2.

Cont  
E 15

120. (Amended) The method of claim 114, wherein said reporter gene substrate is CCF2.

121. (Amended) The method of claim 116, wherein said reporter gene substrate is CCF2.

Sub  
317

122. (Amended) The method of claim 75, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

123. (Amended) The method of claim 81, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

Please add new claims 124 to 138 as below:

014

--124. (New) The method of claim 90, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

Sub  
318

125. (New) The method of claim 94, wherein said GPCR is selected from the group consisting of muscarinic receptors, nicotinic acetylcholine receptors, GABA receptors, glutamate receptors, adrenergic receptors, dopamine receptors and serotonin receptors.

014 126. (New) The method of claim 74, wherein said intracellular calcium indicator is Fura II.

127. (New) The method of claim 78, wherein said intracellular calcium indicator is Fura II.

128. (New ) The method of claim 93, wherein said intracellular calcium indicator is Fura II.

---

129. (New) The method of claim 63, wherein said second heterologous promoter is NFAT.

Sum 3/19  
130. (New) The method of claim 81, wherein said second heterologous promoter is NFAT.

131. (New) The method of claim 94, wherein said second heterologous promoter is NFAT.

132. (New) The method of claim 102, wherein said second heterologous promoter is NFAT.

---

Sum 3/16  
133. (New) The method of claim 63, wherein said method further comprises comparing said change in reporter gene expression detected in step (iii) with a change in reporter gene expression detected in a second control cell line lacking said GPCR detected under the same conditions as in step (iii).

134. (New) The method of claim 71, wherein said method further comprises comparing said change in signal detected in step (iii) with a change in signal detected in a second control cell line lacking said GPCR detected under the same conditions as in step (iii).